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EXAMINER	
ROONEY, NORA MAUREEN	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/590,940	GERACI, DOMENICO	
	Examiner	Art Unit	
	NORA ROONEY	1644	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *04 June 2011*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) *23,33-41,44 and 49-54* is/are pending in the application.

5a) Of the above claim(s) *33-36,40,41 and 49-54* is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *23, 37-39, 44* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

12)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. Applicant's amendment filed on 06/04/2011 is acknowledged.

2. Claims 23, 33-41, 44, 49-54 are pending.

3. Claims 33-36 and 40-41 and 49-54 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected Groups, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on 07/29/2009.

4. Claims 23, 37-39 and 44 are currently under examination as they read on a fusion protein characterized in that it comprises allergens Parj1 and Parj2 of the Parietaria judaica species, in that said allergens lack one or more of the four disulphide bridges present in wild type allergens, at least one in the amino terminal region comprised between amino acid residues 1 and 30 and in that said allergens maintain essentially the same length as wild type allergens; wherein said protein comprises the amino acid sequence of SEQ ID NO:4, pharmaceutical compositions thereof and methods of preparing a pharmaceutical composition.

5. Applicant's response filed on 06/04/2011 stating that the Examiner should look to the prosecution histories of Applications 10/380,002, 10/557,586 and 12/933,390 is noted. Documents that are relevant to the instant application should be listed on an Information Disclosure Statement and filed in the instant application to be considered and printed on the face of any U.S. Patent resulting from the instant application.

6. The following rejections are necessitated by the amendment filed on 06/04/2011.

7. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

8. Claims 23, 37-39 and 44 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 23 recites amino acid residues by number without a reference sequence, making the claims indefinite. Without the inclusion of a SEQ ID NO to the claims, amino acids numbers reads on any amino acids, particularly since the claims are directed to fusion proteins which may comprise any deletion and any number of amino acids from any other protein added anywhere to the protein.

It is noted that Applicant has added SEQ ID NO:4 to claim 23, however the amino acid position numbers do not necessarily refer to amino acids 1 and 30 of SEQ ID NO:4 as currently recited. The claim is currently reciting positions 1 and 30 in reference to the amino terminus of the two allergens, not the amino terminus of the fusion protein sequence.

Furthermore, if the fusion protein of claim 23 is limited to SEQ ID NO:4, it is unclear how the fusion protein "lacks one or more of the four disulphide bridges." It is unclear how the fusion protein of SEQ ID NO:4 could lack "one or more" disulphide bridges. The properties of the fusion protein comprising mutations at residues responsible for disulphide bridges are dependent upon its structure, so SEQ ID NO:4 should have one specific number of disulphide bridges present in the wild type allergens possible from its sequence.

Correction is required.

9. Claim 23 recites the limitation "protein" in line 8. There is insufficient antecedent basis for this limitation in the claim. It is suggested that Applicant amend the claim to recite "fusion protein" in claim 8 instead.

Correction is required.

Claim Rejections - 35 USC § 103

10. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

11. Claims 23, 37-39 and 44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Columbo et al. (IDS filed on 10/20/2010) in view of Columbo et al. (IDS filed on 08/28/2006) Bonura et al. (IDS filed on 08/28/2006) and Pauli et al. (PTO-892 mailed on 04/15/2010; Reference U).

Columbo et al. (IDS filed on 10/20/2010) teaches that Par j 1 and Par j 2 are the two major allergens in Parietaria judaica pollen which are the main cause of allergy in the Mediterranean. Parietaria pollen also contains at least 7 other minor allergens (In particular, second paragraph on page 2780). Par j 1 and Par j 2 allergens exhibit 45% overall homology and

60% homology in the 1-30 region of the proteins known to have one common, discontinuous IgE epitope (In particular, second paragraph on page 2780, first paragraph on page 2784). Colombo et al teaches Par j 1 and Par j2 allergens with substantially the same sequences as 1-102 of SEQ D NO:4 (Parr j2) and 105-243 of SEQ ID NO:4 (Par j I). The reference also teaches loop 1 allergen mutants mutated in amino acids 1-30 of 1-102 of SEQ D NO:4 (Parr j2) and 105-243 of SEQ ID NO:4 (Par j I) for diagnosis and therapy of pollen allergy (In particular, page 2781-2782 'Materials and Methods', sequences in Figures 2-3, whole document). Columbo et al. teaches that mutation of positions C4, C14, C29, and C30 effects structure and substitution with serine at positions 14 and 29 and deletion of position 29 leads to a decrease in IgE binding in this region (In particular, page 2782 first full paragraph, Figure 2). The reference teaches that the development of hypoallergenic molecules with reduced or eliminated IgE binding can be used in allergen therapies (last paragraph), particularly given the importance of these allergens to allergy in the Mediterranean and throughout the world (In particular, second paragraph on page 2780, last paragraph on page 2784). It is noted that 105-243 of SEQ ID NO:4 (Par j I) is the sequence for Par j1 with C4, C29 and C30 mutated to serine and 1-102 of SEQ D NO:4 (Parr j2) is the sequence for Par j 2 with C4, C29 and C30 mutated to serine.

The claimed invention differs from the prior art in the recitation of "a fusion protein characterized in that it comprises allergens Parj1 and Parj2 of the Parietaria judaica species, in that said allergens lack one or more of the four disulphide bridges present in wild type allergens, at least one in the amino terminal region comprised between amino acid residues 1 and 30 and in that said allergens maintain essentially the same length as wild type allergens; wherein said protein comprises the amino acid sequence of SEQ ID NO:4" of claim 23; "a pharmaceutical

composition comprising the fusion protein according to claim 23 and a pharmaceutically acceptable excipient" of claim 37; "the pharmaceutical composition according to claim 37 in the form of a solution, suspension, emulsion, cream, ointment or implant" of claim 38; “and “a method for preparation of the pharmaceutical composition according to claim 37, the method comprising mixing said protein in an immunologically active amount with a pharmaceutically acceptable excipient” of claim 44.

Columbo et al. (IDS filed on 08/28/2006) teaches that the Par j 1 major allergen has been shown to adopt the same structural fold with four disulfide bridges in the following order: Cys4-Cys52, Cys14-Cys29, Cys30-Cys75 and Cys50-Cys91and that the same folding has been shown for Par j 2; The immunodominant IgE epitope is located in the loop l region located between alpha-helixl and alpha-helix2 in the region from amino acids 1 to 30 (In particular, Table 2, page 177). The reference teaches that Cys14-Cys29, Cys30-Cys75 are the cysteine bridges that are most important for IgE binding and allergenicity (In particular, page 177, right column).

Bonura et al. teaches that Par j 1 is a major allergen in Parietaria judaica pollen and a main cause of allergy in the Mediterranean. Par j 1 allergen exhibits a high level of homology with the family of non-specific lipid transfer proteins (In particular, page 33, left column). Bonura et al teaches Par j 1 with substantially the same sequences as amino acids 105-243 of SEQ ID NO:4. The reference also teaches mutants that disrupt the cysteine bridges at C14/C29, C30/C75 and C4/C52 by mutation of those cysteine residues with serine for in vivo pharmaceutical use in a pharmaceutically acceptable excipient (In particular, page 37, right column, 'Materials and Methods', whole document). Bonura et al. teaches that mutation of positions C4, C52, C14, C29, C30 and C75 effect structure and leads to a loss of IgE binding in

this region (In particular, whole document). The reference teaches that the development of hypoallergenic molecules with reduced or eliminated IgE binding can be used in allergen therapies (In particular, discussion). It is noted that 105-243 of SEQ ID NO:4 (Par j I) is the sequence for Par j1 with C4, C29 and C30 mutated to serine.

Pauli et al. teaches that dimer and trimer multimer fusion proteins of Bet v 1 in pharmaceutical compositions exhibited reduced skin reactions as determined by in vivo intradermal and skin prick testing (In particular, whole document). The reference also teaches that the dimer and trimer fusion Bet v 1 molecules had retained IgE binding capacity and fold, but microaggregation led to decreased effector cell activation (In particular, page 1081, second full paragraph). The reference suggested that pharmaceutical compositions comprising the multimers for the treatment of allergy should also contain adjuvants to prevent spreading of molecules and to decrease systemic reactions (In particular, page 1082, first paragraph).

It would have been obvious to one of ordinary skill in the art at the time of invention to combine the teachings of both Columbo et al. references and Pauli et al. produce a multimer fusion protein comprising Par j 1 and Par j 2 to treat allergies because Par j 1 and Par j 2 are the major allergens of Parietaria pollen. It would have been obvious to only include these two allergens since they are the two major allergens and it is desirable to produce pharmaceutical compositions which only comprise the most important allergens without the confounding effects of the seven minor allergens and other components normally present in pollen allergen extracts. By combining Par j 1 and Par j 2 into a single molecule, the molar ratio of the two allergens will be constant, thus providing a controlled dosage of both allergens to patients for optimal

immunotherapy use. Because Pauli et al. teaches that dimerization and trimerization of allergens does not lead to a change in the conformation of the allergen fold and Columbo et al. teaches that the 1-30 IgE epitope of Par j1 and Par j 2 is a conformational, discontinuous epitope, it would also have been obvious to perform mutational analysis at the positions taught by Columbo et al. to generate a Par j1/ Par j2 multimer protein with reduced IgE binding at that epitope. One would be motivated to do this because Columbo et al teaches that it is an important IgE epitope and because the multimer is being generated for in vivo use. It is obvious to combine two compositions which are known to have the same use. One of ordinary skill in the art at the time of invention would have been motivated to perform mutations to arrive at SEQ ID NO 4 for in vivo allergy therapy use, which may further contain an adjuvant because such a molecule would be expected to exhibit reduced IgE binding in addition to reduced effector cell activation when used in vivo to treat allergies. It would be obvious to one of ordinary skill in the art at the time the invention was made to combine the compositions of Columbo et al. and Pauli et al. because it is prima facie obvious to combine two compositions each of which is taught by prior art to be useful for same purpose in order to form third composition that is to be used for the very same purpose. The idea of combining them flows logically from their having been individually taught in prior art. In re Kerkhoven, 205 USPQ 1069, CCPA 1980. See MPEP 2144.06.

From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expection of success in producing the claimed invention. Therefore, the invention as a whole was *prima facie* obvious to one of ordinary skill in the art at

the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

Applicant's arguments filed on 06/04/2011 have been fully considered, but are not found persuasive.

Applicant argues:

"Applicants' invention provides a fusion protein for use in specific immunotherapy treatment of allergies, namely allegenic constructs having a decreased ability to bind and activate IgE, but retaining (or even improving) the immunogenic capability of the wild type allergens. The decrease in binding capability is shown by low scores (%) of inhibition in a binding-inhibition ELISA assay.

Since more than one antigen actively contributes to eliciting an allergic response (e.g., both Parjl and Parj2 contribute to inducing *Parietaria* allergy), effective treatment of allergy should be obtained using both antigens. Here, Applicants' invention uses a fusion protein comprising amino acid sequences of two different allergens that were modified to acquire desirable properties, specifically the amino acid sequence SEQ ID NO: 4.

The binding properties of the fusion protein of claim 28 (see PjEDcys in Fig. 2) is clearly shown by the results reported in the present examples. Fusion protein PjEDcys exhibits a dramatically reduced binding capability to IgE (thus, a reduced capability of interacting with IgE and activating an immune response) as compared to the corresponding wild type heterodimer (see Fig. 5). Fig. 5 reports an average inhibition for PjEDcys of about 7.3% against 62% for the wild type allergen. PjEDcys also exhibits a reduced binding capability to IgE compared to the single muteins (monomers) PjA, PjB, PjC, and PjD[1]. Fig. 9 shows an inhibition of 14% for the best of these muteins as shown by PjC that is, however, twice as high as PjEDcys.

PjEDcys also exhibits a reduced binding capability to IgEs as compared to the mixture of wild type rParjl and rParj2 (average inhibition 76.5%), rParjl only (average inhibition 40.1%), or rParj2 only (average inhibition 61.1 %) as reported in Fig. 8. This reduced binding to IgE is unexpectedly accompanied by enhanced immunogenic capability as shown in Fig. 7. The results reported in Fig. 7D demonstrate that PjEDcys is capable of stimulating CD3+ cells more efficiently than a mixture of separate allergens Parjl and Parj2 (which, by the way, is shown to exhibit the highest binding ability to IgE). Therefore, the claimed fusion protein (e.g., PjEDcys) is a patentable improvement over the individual proteins separately.

These results are completely unexpected from documents cited in the rejections below. Moreover, the results reported in and derivable from the present specification are confirmed by the recent publication of Bonura et al. (Int'l Arch. Allergy Immunol. 142: 274-284, 2007; previously made of record).

It was alleged that Colombo (1998) teaches mutations in positions C4, C52, C14, C29, C30 and C75 lead to a loss of IgE binding in this region. But this is incorrect. It is clear from the section "Epitope Mapping" (see bottom of page 2781) that the sole Cys residues considered in Colombo are those in the 1-30 fragment. Actually, Colombo prepared only deletions or substitutions in positions 30, 29, 4 and 14 (see Fig.2A): deletion C30 (pPJ1.5), deletions C29 and C30 (pPJ1.6), substitution C4S (pPJ1.7), substitution C29S (pPJ1.8), and substitution C14S (pep3). Of these, pPJ1.5 (deletion C30) and pPj1.7 (substitution

C4S) are said to be still capable of bindinq IqE (page 2781, last line, and page 2782, lines 1-4). Colombo also reported other mutations that result in loss of IgE binding, namely K21, K23, E24 and K27.

Therefore, Colombo does not teach or make obvious any mutation in position 52 or 75 as alleged by the Examiner. Moreover, Colombo does not teach or make obvious that mutations in position 30, 29, 14 or 4 result in loss of IgE binding. On the contrary, Colombo discloses that mutations in position 30 or 4 do not affect IgE binding. Colombo also does not disclose, or even make obvious, that the disruption of disulphide bridges is the sole means to inhibit IgE binding. Instead, a range of possible mutations including cysteine, lysine (K), and glutamic acid (E) residues are disclosed. Further, Colombo does not teach or make obvious any specific combination of modifications, such as the combination C4, C29 and C30 characterizing the fusion protein PjEDcys (see Fig. 2 and SEQ ID NO: 4). Rather, Colombo teaches away from constructs comprising modification in positions C30 or C4 because pPj1.5 (C30) and pPJ17 (C4) are still capable of bindinq IgE. Finally, Colombo fails to teach or make obvious any dimeric fusion protein that comprises muteins of two different allergens (e.g., Parjl and Parj2). This difference was acknowledged by the Examiner.

Bonura corresponds to the disclosure of WO 02/20790 and teaches muteins of only the Parjl allergen. The IgE binding activity of any single mutein PjA, PjB, PjC and PjD is analyzed, and the results are the same as those reported in Fig. 9 of the present application at issue. Bonura, like Colombo, does not teach or make obvious any heterodimer fusion protein comprising muteins of two different allergens: i.e., Parjl and Parj2.

The Examiner asserts, however, that Pauli teaches the improved efficacy of a dimeric form of an allergen and would, therefore, suggest the construction of a dimeric fusion protein comprising the Parjl and Parj2 allergens as mutated according to Colombo or Bonura. This conclusion is clearly wrong.

Firstly, Pauli's experimental work relates to Bet v-1 allergen and its derivatives. But Bet v-1 and Bet v-2 are birch *(betullaceous)* tree pollen allergens that are taxonomically not related to the plants and ns-LTP allergens of Applicants' invention.

Pauli's disclosure appears to be completely immaterial as regards the claimed invention, since the results reported therein does not suggest anything concerning the activity of a group of allergens (ns-LTP) totally unrelated to birch tree allergens. There is also no other evidence of record establishing a reasonable expectation of success that one of ordinary skill in the art would have predicted the effects obtained when the modifications reported by Pauli are applied to ns-LTP allergens, nor would the same inhibition of IgE binding observed by Pauli be expected for a totally different allergen group.

Secondly, Pauli discloses derivatives (i.e., either fragments or dimers or trimers) of a single allergen: Bet v 1. Thus, one of ordinary skill in the art, by combining Colombo with Bonura and Pauli, would have prepared, at best, an allergen derivative, perhaps in modified form, free of one or more disulphide bridges, but always deriving from only one allergen: either Parjl or Parj2. Therefore, even combining the cited documents together, one of ordinary skill in the art would not have concluded that it was obvious to prepare a heterodimeric fusion protein comprising different allergens: i.e., both Parjl and Parj2.

Finally, Pauli not only does not make obvious the claimed invention, but actually teaches away from preparing and assaying any dimeric fusion protein. One of ordinary skill in the art is cautioned of the risk of anaphylactic side effects induced by injecting Bet v-1 wild type allergen or a derivative thereof (see page 1082, right hand column, middle paragraph, starting with "That hypoallergenic molecules can be injected..."). For this reason, Pauli specifically admits, "We did not include Bet vl dimer in the intradermal tests because it proved to have a higher skin test reactivity than Bet vl trimer in the skin pick test" (page 1082, left hand column, last paragraph). For this reason, Pauli concludes, "It is planned to prepare vaccine which consist of the two rBet v-1 fragments or rBet v-1 trimer adsorbed to AI(OH)3" (page 1082, last line,

> et seq.). Therefore, one of ordinary skill in the art reading Pauli would not have found it obvious to prepare a fusion protein comprising a Bet v-1 dimer.
>
> If a modification proposed by the Examiner would render a prior art invention inoperable for its intended purpose, then the cited prior art effectively teaches away from the proposed modification and fails to establish a prima facie case of obviousness. See *In re Gordon,* 221 USPQ 1125 (Fed. Cir. 1984). Moreover, if the proposed modification would change the principle of operation of the prior art invention being modified, then the cited prior art also fails to establish a prima facie case of obviousness. See *In re Ratti,* 123 USPQ 349 (CCPA 1959). Thus, Colombo and Pauli cannot be relied upon to establish a case of prima facie obviousness because the operation of Applicants' invention is incompatible with the teachings of the cited documents.
>
> Finally, the Examiner is required to consider whether the improvement obtained by the present invention is more than the predictable use of prior art elements according to their established functions. See *KSR* at 1396. In Applicants' invention, the improvement in the use of the claimed fusion protein in specific immunotherapy would not have been predicted from the cited documents."

It is the Examiner's position that, as evidenced by the rejection mailed on 01/04/2001, it has never been alleged that Columbo (1998) teaches mutations in positions C4, C52, C14, C29, C30 and C75 lead to a loss of IgE binding in this region (see above rejection which is an exact copy of the rejection mailed on 01/04/2011), contrary to Applicant's assertion. It is however true that Bonura et al. teaches that mutations of positions C4, C52, C14, C29, C30 and C75 effect structure and lead to a loss of IgE binding. Applicant's argument for what Columbo (1998) teaches is similar to what the Examiner wrote in the rejection mailed on 01/04/2011 and copied above.

It is the Examiner's position that Colombo need not disclose or make obvious that the disruption of disulphide bridges is the sole means to inhibit IgE binding nor that any specific combination of modifications as that is not required for the instant rejection. Colombo does not teach away from constructs comprising modification in positions C30 or C4, though they are still capable of binding IgE because Columbo et al. teaches that mutations at these positions effect structure, which is the point of making mutations at disulphide bridges. Columbo taught that

mutations at positions C30 and C4 successfully change the structure of the allergen and that teaching alone is sufficient and does not teach away from the invention.

Neither of the Columbo references nor the Bonura reference is being used to teach dimeric fusion proteins of Parj 1 and Parj2, as that teaching is supplied by Pauli et al. Bet v 1 allergens are analogous art and the teachings of Pauli et al. using Bet v 1 allergens can easily be applied to other pollen allergens. The taxonomic relationship is not the standard by which one judges the applicability of Pauli et al. to the instant invention. Both are pollen allergens and the concepts of Pauli et al. are easily applied to Parietaria judaica and other pollen allergens. Contrary to Applicant's assertion, one of ordinary skill in the art would certainly apply the teaching of Pauli et al. to other pollen allergens. There is nothing unique about Bet v 1 which would make the teachings of Pauli et al. specific to Bet v 1 or only to other birch pollen allergens. The field of allergens and allergen immunotherapy methods teach modification methods that applicable to all allergens as the mechanism of allergy, IgE binding to allergens, is common to them all by definition. In the same way, methods to decrease IgE binding to a protein by changing the structure is common to all allergens as well. Bet v1 is a protein that binds IgE and so are Parj1 and Parj2. Therefore, the teachings of Pauli et al. are easily applied to the other references. Pauli et al. is relied upon for its teaching of dimers and trimers. Pauli et al. does not teach that dimerization and trimerization could not be applied to two different protein allergens. The same protein can just as easily be dimerized or timerized as two different proteins, particularly in view of the fact that Parj1 and Parj2 are 45% homologous. The motivation to use Parj1 and Parj2 comes from both Columbo references which teach that Parj1 and Parj2 are the

two major allergens from Parietaria judaica. It would be obvious to use both major allergens in allergen immunotherapy methods for Parietaria judaica allergies.

It is noted that the "comprising" language of the instant claims does not exclude the possibility that a trimer is encompassed by the instant claims. Furthermore, the reference teaches that both the dimer and trimer exhibited greatly reduced basophil histamine release and did not induce wheal or flare reactions up to 1000 micrograms per ml (In particular, page 1077, left column). The reference teaches on page 1082, left column, that the dimer was not included in the intradermal tests because it exhibited higher skin test reactivities than the trimer in the skin prick test. However, this teaching does not teach away from the use of a dimer. (In particular, see Figure 2). There is nothing in the Pauli et al. reference to suggest that the dimer is inoperable, contrary to Applicant's assertion.

It remains the Examiner's position that the Columbo references and the Bonura reference together provide motivation to change the conformational IgE binding epitopes of Par j 1 and Par j 2. The references highlight the important residues for structure and IgE binding and the references make it obvious to mutate and/or delete one or more of the cysteine residues involved in maintaining structure and IgE binding. One would reasonably expect that dimers and trimers of the mutated allergens would also decrease allergenicity and basophil histamine release, given the teachings of Pauli et al. Therefore, one of ordinary skill in the art would have a high expectation of success in generating the recited hypoallergenic fusion molecules.

12. Claims 23, 37-39 and 44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Vrtala et al. (PTO-892 mailed on 04/15/2010; Reference V) in view of Colombo (IDS filed on

08/28/2006), Columbo et al. (IDS filed on 10/20/2010) and Bonura et al. (IDS filed on 08/28/2006).

Vrtala et al. teaches recombinant multimeric protein allergen such as dimer and trimer of major birch pollen allergen Bet vl, (In particular, page 2045 and whole document). The recombinant trimer consisting of three covalently linked copies of the allergens is useful for inducing IgG antibodies in vivo (pharmaceutical composition mixed in solution, comprising pharmaceutically acceptable excipient) and blocking IgE binding to Bet vl and related allergens, (In particular, abstract and page 2047).

The claimed invention differs from the prior art in the recitation of " a fusion protein characterized in that it comprises the amino acid sequences of different allergens belonging to the non-specific Lipid Transfer Protein (ns-LTPs) family, in that said sequences lack one or more of the four disulphide bridges present in the sequences of the wild type allergens, at least one in the amino terminal region comprised between amino acid residues 1 and 30 and in that said sequences maintain essentially the same length as the sequences of wild type allergens" of claim 23; "characterized in that the amino acid sequence of each of the allergens is independently mutated by elimination or substitution of one or more cysteine residues involved in the formation of a disulphide bridge" of claim 24; "characterized in that it comprises allergens Parjl and Parj2 of the *Parietaria judaica* species" of claim 25; "characterized in that the amino acid sequence of each of the allergens is independently mutated by elimination or substitution of one or more cysteine residues in positions corresponding to the positions 4, 14, 29, 30, 50, 52, 75 and 91 of the amino acid sequence of Parj 1 and/or Parj2 allergen" of claim 26; "characterized in that it contains amino acid sequences of Parjl and Parj2 allergens, both independently modified by

substitution of cysteine residues with Asn, Ser, Thr, lie, Met, Gly, Ala, Val, Gln or Leu residues in positions 29 and 30 or 4, 29 and 30 or 29, 30, 50, 52" of claim 27 and "comprising the amino acid sequence SEQ ID NO: 4" of claim 36.

Columbo et al. (IDS filed on 10/20/2010) teaches that Par j 1 and Par j 2 are the two major allergens in Parietaria judaica pollen which are the main cause of allergy in the Mediterranean. Parietaria pollen also contains at least 7 other minor allergens (In particular, second paragraph on page 2780). Par j 1 and Par j 2 allergens exhibit 45% overall homology and 60% homology in the 1-30 region of the proteins known to have one common, discontinuous IgE epitope (In particular, second paragraph on page 2780, first paragraph on page 2784). Colombo et al teaches Par j 1 and Par j2 allergens with substantially the same sequences as 1-102 of SEQ D NO:4 (Parr j2) and 105-243 of SEQ ID NO:4 (Par j I). The reference also teaches loop 1 allergen mutants mutated in amino acids 1-30 of 1-102 of SEQ D NO:4 (Parr j2) and 105-243 of SEQ ID NO:4 (Par j I) for diagnosis and therapy of pollen allergy (In particular, page 2781-2782 'Materials and Methods', sequences in Figures 2-3, whole document). Columbo et al. teaches that mutation of positions C4, C14, C29, and C30 effects structure and substitution with serine at positions 14 and 29 and deletion of position 29 leads to a decrease in IgE binding in this region (In particular, page 2782 first full paragraph, Figure 2). The reference teaches that the development of hypoallergenic molecules with reduced or eliminated IgE binding can be used in allergen therapies (last paragraph), particularly given the importance of these allergens to allergy in the Mediterranean and throughout the world (In particular, second paragraph on page 2780, last paragraph on page 2784). It is noted that 105-243 of SEQ ID NO:4 (Par j I) is the sequence

for Par j1 with C4, C29 and C30 mutated to serine and 1-102 of SEQ D NO:4 (Parr j2) is the sequence for Par j 2 with C4, C29 and C30 mutated to serine.

Columbo et al. (IDS filed on 08/28/2006)teaches that the Par j 1 major allergen has been shown to adopt the same structural fold with four disulfide bridges in the following order: Cys4-Cys52, Cys14-Cys29, Cys30-Cys75 and Cys50-Cys91and that the same folding has been shown for Par j 2; The immunodominant IgE epitope is located in the loop l region located between alpha-helixl and alpha-helix2 in the region from amino acids 1 to 30 (In particular, Table 2, page 177). The reference teaches that Cys14-Cys29, Cys30-Cys75 are the cysteine bridges that are most important for IgE binding and allergenicity (In particular, page 177, right column).

Bonura et al. teaches that Par j 1 is a major allergens in Parietaria judaica pollen and a main cause of allergy in the Mediterranean. Par j 1 allergen exhibits a high level of homology with the family of non-specific lipid transfer proteins (In particular, page 33, left column). Bonura et al teaches Par j 1 with substantially the same sequences as amino acids 105-243 of SEQ ID NO:4. The reference also teaches mutants that disrupt the cysteine bridges at C14/C29, C30/C75 and C4/C52 by mutation of those cysteine residues with serine for in vivo pharmaceutical use in a pharmaceutically acceptable excipient (In particular, page 37, right column, 'Materials and Methods', whole document). Bonura et al. teaches that mutation of positions C4, C52, C14, C29, C30 and C75 effect structure and leads to a loss of IgE binding in this region (In particular, whole document). The reference teaches that the development of hypoallergenic molecules with reduced or eliminated IgE binding can be used in allergen therapies (In particular, discussion). It is noted that 105-243 of SEQ ID NO:4 (Par j I) is the sequence for Par j1 with C4, C29 and C30 mutated to serine.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute the Par j 1 and Par j2 allergens taught by the Colombo references and Bonura et al. in the major birch pollen allergen Bet vl dimers and trimers of Vrtala et al because Vrtala et al. teaches that the dimers and trimers are useful for diagnosis and/or treating allergy. Both Colombo et al. references teache that Par j1 and Pa j 2 can themselves be useful for diagnosis and therapy of Parietaria pollen allergy, so it would be obvious to generated multimer fusions of the allergens for diagnosis and therapy as well. It would have been obvious to mutate both Par j 1 and Par j 2 in the same cysteine residues since Columbo et al. (IDS filed 10/20/2010) teaches that Par j 1 and Par j 2 allergens exhibit 45% overall homology and 60% homology in the 1-30 region of the proteins known to have one common, discontinuous IgE epitope.

From the reference teachings, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was *prima facie* obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the reference, especially in the absence of evidence to the contrary.

Applicant's arguments filed on 06/04/2011 have been fully considered, but are not found persuasive.

Applicant argues:

> "Applicants' invention provides a fusion protein for use in specific immunotherapy treatment of allergies, namely allegenic constructs having a decreased ability to bind and activate IgE, but retaining (or even improving) the immunogenic capability of the wild type allergens. The decrease in binding capability is shown by low scores (%) of inhibition in a binding-inhibition ELISA assay.

Since more than one antigen actively contributes to eliciting an allergic response (e.g., both Parjl and Parj2 contribute to inducing *Parietaria* allergy), effective treatment of allergy should be obtained using both antigens. Here, Applicants' invention uses a fusion protein comprising amino acid sequences of two different allergens that were modified to acquire desirable properties, specifically the amino acid sequence SEQ ID NO: 4.

The binding properties of the fusion protein of claim 28 (see PjEDcys in Fig. 2) is clearly shown by the results reported in the present examples. Fusion protein PjEDcys exhibits a dramatically reduced binding capability to IgE (thus, a reduced capability of interacting with IgE and activating an immune response) as compared to the corresponding wild type heterodimer (see Fig. 5). Fig. 5 reports an average inhibition for PjEDcys of about 7.3% against 62% for the wild type allergen. PjEDcys also exhibits a reduced binding capability to IgE compared to the single muteins (monomers) PjA, PjB, PjC, and PjD[2]. Fig. 9 shows an inhibition of 14% for the best of these muteins as shown by PjC that is, however, twice as high as PjEDcys.

PjEDcys also exhibits a reduced binding capability to IgEs as compared to the mixture of wild type rParjl and rParj2 (average inhibition 76.5%), rParjl only (average inhibition 40.1%), or rParj2 only (average inhibition 61.1 %) as reported in Fig. 8. This reduced binding to IgE is unexpectedly accompanied by enhanced immunogenic capability as shown in Fig. 7. The results reported in Fig. 7D demonstrate that PjEDcys is capable of stimulating CD3+ cells more efficiently than a mixture of separate allergens Parjl and Parj2 (which, by the way, is shown to exhibit the highest binding ability to IgE). Therefore, the claimed fusion protein (e.g., PjEDcys)is a patentable improvement over the individual proteins separately.

These results are completely unexpected from documents cited in the rejections below. Moreover, the results reported in and derivable from the present specification are confirmed by the recent publication of Bonura et al. (Int'l Arch. Allergy Immunol. 142: 274-284, 2007; previously made of record).

Vrtala's disclosure is not substantially different from Pauli. Vrtala relates to Bet v- 1 allergen. As already explained above, Bet v-1 is a birch tree *(betullaceous)* pollen allergen and is not related to the ns-LTP allergens of the present application. The cited document appears to be completely immaterial as regards the claimed invention, since the results reported therein does not suggest anything concerning the activity of a group of allergens (ns-LTP) totally unrelated to birch tree allergens. There is also no other evidence of record establishing a reasonable expectation of success that one of ordinary skill in the art would have predicted the effects obtained when the modifications
reported by Vrtala are applied to ns-LTP allergens, nor would the same inhibition of IgE binding observed by Vrtala be expected for a totally different allergen group.

Further, Vrtala discloses dimers or trimers of a single allergen: Bet v 1. Thus, one of ordinary skill in the art, by combining Vrtala with Colombo and Bonura, would have prepared, at best, an allergen derivative, perhaps in modified form, free of one or more disulphide bridges, but always deriving from one allergen: either Parjl or Parj2. Therefore, even combining the three cited documents together, one of ordinary skill in the art would not have concluded from the evidence of record that it was obvious to prepare a heterodimeric fusion protein comprising different allergens: i.e., both Parjl and Parj2.

In any case, Vrtala, like Pauli, focuses on a trimer rather than a dimer of Bet vl allergen. This is evident not only from the title, but also from the section "Conclusions" that are totally silent on any reason to prepare the dimer. The reason resides in the fact that the dimer is much less preferred than the trimer for use because the former induces a higher skin reaction and allergic activity when compared to the latter. This is shown in the results reported in section "Recombinant Bet vl trimer exhibits profoundly reduced allergic activity" on page 2045. The mean wheal diameters of the skin reaction area (at 10 tJg/ml) is 4.1 + 2.4 (dimer) vs. 0.7 + 1.2 (trimer) and (at 100 tJg/ml) 7.3 + 2.5 (dimer) vs. 3.6 + 2.1 (trimer). These results confirm the previously discussed prior art's teaching away from Applicants' invention, and would have further contributed to dissuading one of ordinary skill in the art from considering the dimeric form as a valid tool for preparing a vaccine for immunotherapy.

> If a modification proposed by the Examiner would render a prior art invention inoperable for its intended purpose, then the cited prior art effectively teaches away from the proposed modification and fails to establish a prima facie case of obviousness. See *In re Gordon,* 221 USPQ 1125 (Fed. Cir. 1984). Moreover, if the proposed modification would change the principle of operation of the prior art invention being modified, then the cited prior art also fails to establish a prima facie case of obviousness. See *In re Ratti,* 123 USPQ 349 (CCPA 1959). Thus, Vrtala and Colombo cannot be relied upon to establish a case of prima facie obviousness because the operation of Applicants' invention is incompatible with the teachings of the cited documents.
>
> Finally, the Examiner is required to consider whether the improvement obtained by the present invention is more than the predictable use of prior art elements according to their established functions. See *KSR* at 1396. In Applicants' invention, the improvement in the use of the claimed fusion protein in specific immunotherapy would not have been predicted from the cited documents.
>
> Withdrawal of the Section 103 rejections is requested because the claims would not have been obvious to one of ordinary skill in the art when this invention was made."

It is the Examiner's position that the teachings of Vrtala et al. with regard to Bet v1 allergens are highly applicable to the teachings of Parj1 and Parj2. The pollen source does not impact the fact that all protein allergens can be modified to change the structure in order to reduce IgE binding. It is not "completely immaterial" to the instant invention, as alleged by Applicant. Contrary to Applicant's assertion, there is ample evidence throughout the references to suggest that allergens with modified structures exhibit reduced IgE binding.

The dimerization and trimerization process of Vrtala et al. is not dependent upon using a single allergen and can easily be performed using two different allergens linked in the same manner. The linking of the allergens is what makes the multimers hypoallergenic. The combination of both Columbo references provides the motivation to linke two different proteins since Parj1 and Parj2 are the two major Parietaria judaica pollen allergens, particularly since they are highly homologous. In addition, it is noted that the "comprising" language does not exclude the use of a trimer. Furthermore, the dimers are prepared in Vrtala et al. and that teaching alone is sufficient motivation to prepare Parj1 and Parj2 dimers. Being less preferred does not amount

to teaching away. The trimer was particularly hypoallergenic, but that does not mean that one would not be motivated to make a dimer, particularly if two different allergens are dimerized and useful for dosage of the two major Parietaria judaica allergens.

It remains the Examiner's position that the Columbo references and the Bonura reference together provide motivation to change the conformational IgE binding epitopes of Par j 1 and Par j 2. The references highlight the important residues for structure and IgE binding and the references make it obvious to mutate and/or delete one or more of the cysteine residues involved in maintaining structure and IgE binding. One would reasonably expect that dimers and trimers of the mutated allergens would also decrease allergenicity and basophil histamine release, given the teachings of Vrtala et al. Therefore, one of ordinary skill in the art would have a high expectation of success in generating the recited hypoallergenic fusion molecules.

13. No claim is allowed.

14. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

15. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Nora M. Rooney whose telephone number is (571) 272-9937. The examiner can normally be reached Monday through Friday from 8:30 am to 5:00 pm. A

message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached on (571) 272-0735. The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

August 29, 2011

/Nora M Rooney/

Primary Examiner, Art Unit 1644